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Effect of Cooling, and Freezing on the Digestibility of Debranched Rice Starch and Physical Properties of the Resulting Material

Ten percent non-waxy and waxy starch suspensions were debranched with pullulanase followed by heating and cooling (1 °C) to crystallize and/or gel. Products with a range of textures can be made depending on the type (waxy and non-waxy) of starch used. The water holding capacity was 35% and 84% for waxy and non-waxy cooled debranched starch, respectively, at 4 h of cooling and did not change. The hardness of the debranched waxy and non-waxy starch continued to increase beyond 24 h up to 45 g and 245 g of force, respectively. The particle size of precipitates of non-waxy and waxy debranched starch was 45 µm and 4 µm after 4 h of cooling and did not change. Cooling of debranched non-waxy starch at 1 °C for 12 h without agitation decreased digestibility by 59%; with stirring digestibility decreased by 42% after 24 h of cooling. Freezing of debranched cooled waxy and non-waxy starch does not effect the decreases in digestibility. Particle size of debranched, cooled/freeze-thawed, dried, and milled starch affects digestibility

Keywords: Slowly digestible starch; Resistant starch; Pullulanase; Cooling

1 Introduction

Intrinsic factors affecting the rate of starch digestion in foods are the physical form of food, shape and crystal structure of the starch granule, recrystallization and retrogradation, amylose-lipid complexes, native α-amylase inhibitors and non-starch polysaccharides [1]. For nutritional purposes, starch is classified into three types based on these intrinsic factors: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [1]. Resistant starch can be further classified into four types: Type I RS (physically inaccessible starch)[1], Type II RS (resistant starch granules) [1], Type III RS (retrograded starch)[1] and Type IV RS (chemically/heat treated starch)[2].

Nutritional properties of starch may be very important for treatment and prevention of several diseases. Elevated plasma glucose or insulin levels after a glucose load have been associated with non-insulin dependent diabetes [3] and cardiovascular diseases [4]. Low glycemic index foods or foods containing SDS maybe helpful in controlling and preventing these diseases. Additionally, such foods may be advantageous to satiety, physical performance, improved glucose tolerance, reduced blood lipid levels in both healthy individuals and those with hyperlipidemia, and have a reduced cariogenic potential [5].

A process for making slowly digestible starch was reported. Cooked non-waxy and waxy starch suspensions (10% w/w) were debranched with pullulanase followed by heating and cooling (1 °C) to form products with a mixture of rapidly digestible starch (RDS), slowly digestible (SDS) and resistant starches (RS). In a previous unreported paper [6] the effect of cooling temperature and debranching enzyme concentration on the formation of RDS, SDS, and RS has been described. We found that an enzyme content of 10% and cooling at 1 °C was optimum for forming reduced digestibility starch. It was also found that cooked non-waxy debranched starch formed a milky white gel with fat like consistency and waxy starch formed a milky white suspensions with precipitates on cooling at 1 °C. In order to use this modified starch in food products its functional properties have to be understood. Our first objective is to study the effect of the time interval of cooling at 1 °C on water holding capacity, texture, particle size, and digestibility. The second objective is to find out if stirring a debranched starch suspension during cooling at 1 °C does also reduce its digestibility or not. The question is "can the debranched starch chains form aggregates on stirring and consequently give a reduction of digestibility or not?" The third objective is to find out if freezing debranched starch at –40 °C prevents the aggregation of starch chains by reducing chain mobility, resulting in lower amount of SDS. The effect of particle size of the dried

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retrograded debranched starch on digestibility was also studied.

2 Materials and Methods

Non-waxy and waxy starch was cooked and debranched. Three experiments were conducted on the same debranched starch. One set of sample of debranched non-waxy and waxy starch was cooled at 1 °C without stirring, and another set was stirred at 1 °C. The water holding capacity, texture and particle size of the precipitates was studied for the non-stirred samples. Digestibility of stirred and non-stirred samples over various time intervals of cooling was compared. For the last experiment, a set of debranched non-waxy and waxy starch samples were removed at 0–24 h of cooling at 1 °C without stirring followed by freezing at –40 °C. The effect of freezing debranched samples on their digestibility was studied. The dried debranched non-waxy sample cooled for 24 h and frozen was used to study the effect of particle size on digestibility.

2.1 Effect of cooling debranched starch on physical properties of resulting material

Waxy (100% amylopectin) and non-waxy (21% amylose, 79% amylopectin) rice starches were obtained from A&B Ingredients, Inc. (Marion, MA). Waxy and non-waxy starch solutions (100%, db) were cooked and cooled to 57.5 °C. The pH of the solutions was adjusted to 4.8–4.9. Ten percent by weight of pullulanase (200 PUN/mL, 1.25 g/mL (density), Novo Nordisk, Franklinton, NC), per dry weight of starch, was added to the cooked starch and the solutions were stirred for 24 h. The debranched starch solutions were autoclaved at 121 °C for 30 min followed by cooling to room temperature in an ice bath, then refrigerated at 1 °C. Gel forming capacity, texture, digestibility, and particle size analysis were conducted on the samples at various time intervals.

2.2 Effect of time of cooling and agitation on digestibility of debranched starch during cooling

Ten percent non-waxy and waxy debranched starch was made as described in Section 2.1. Each batch of debranched non-waxy and waxy starch was continuously stirred during cooling at 1 °C using a Glass-Col (Glass-Col, Terre Haute, IN) stirrer with paddle attachment set at 133 rpm. Samples were withdrawn at 0, 4, 8, and 24 h time intervals during stirring and cooling. Samples were tested for digestibility using α-amylase, and the percentage of maltose released after 1 h was compared to debranched starch samples which were not stirred.

2.3 Effect of cooling debranched non-waxy and waxy starch from 0 to 24 h followed by freezing on digestibility

Ten percent non-waxy and waxy debranched starch was made as described in Section 2.1. Samples were frozen at –40 °C after being stored at 0 to 24 h of cooling at 1 °C. Frozen samples were thawed after 48 h, the debranched starch precipitated and a clear supernatant was formed. The precipitated starch was isolated by repeated washing over filter paper and dried at 60 °C in a hot air oven. Dried starch was reduced in particle size using a Wiley mill with an 80 mesh screen. Samples were tested for digestibility using α-amylase and the percentage of maltose released at various time intervals was compared.

2.4 Effect of particle size on digestibility

Non-waxy debranched starch was cooled for 24 h, frozen, thawed, washed and dried as described in Section 2.3. The dried starch was passed through 20, 30, 60 and 80 mesh screens using a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ) and the digestibility of each fraction was measured using α-amylase. Percentage of maltose released after 12 and 24 h was compared. The particle size was measured using a Coulter particle size analyzer.

3 Analytical Methods

3.1 Degree of debranching

The degree of debranching at various time intervals was followed using the iodine affinity method [7] and gel permeation chromatography [8]. A 10-mL aliquot was withdrawn from each batch undergoing debranching using pullulanase. Aliquots were removed at 0, 4, 8, 12, 18 and 24 h intervals and dispensed into a 20-mL test tube. The test tube was placed in a boiling water bath for 10 min to stop the enzymatic activity. Samples were tested for degree of debranching.

3.2 Water holding capacity

Debranched starch solutions made as described in Section 2.1 were weighed and placed in centrifuge tubes before being refrigerated at 1 °C. Tubes were removed at various time intervals (0, 4, 8, 12, 18 and 24 h) and centrifuged at $3000 \times g$ for 20 min at 20 °C. The aqueous phase was removed and the precipitate was weighed. Initially the starch solution separated into precipitated starch and supernatant which was non-precipitated soluble starch. After several hours of cooling the supernatant was composed of only water with no dissolved starch.

$$\text{Water holding capacity} = \frac{\text{weight of precipitate [g]} \times 100}{\text{weight of wet sample [g]}}$$

3.3 Texture analysis

Debranched starch solutions made as described in Section 2.1 (approx. 43 g) were placed in containers (4.5 cm in diameter × 4.0 cm in height) with lids, and refrigerated at 1 °C. Containers were removed at various time intervals (0, 4, 8, 12, 18 and 24 h). Texture analysis was performed using the Stevens-Farnell QTS25 Materials Evaluation System (Michael G. Brown and Associates, Inc., Newton, PA) in conjunction with the QTS25 Profile Software for Windows. Test conditions consisted of a 2.54 cm diameter × 35 cm height cylindrical probe, a trigger point of 1.0 g, test speed of 120 mm/s, and a target value of 10 g. Fracture force/hardness and adhesive force were measured.

3.4 Digestibility

Two hundred milligrams of debranched samples were withdrawn and placed into test tubes. Phosphate buffer (15 mL, 0.5 M, pH 6.89) was added to each tube followed by vortexing. The in vitro digestibility of the starch solutions was determined by measuring the rate of starch hydrolysis by 1% porcine α-amylase (Type VI-B from porcine pancreas, 29 units of α-amylase/mg of solid at pH 6.9, 1 unit liberating 1.0 mg of maltose in 3 min, E.C. 3.2.1.1, Sigma Chemicals, St. Louis, MO) reconstituted in 0.9% NaCl. The reconstituted α-amylase was aliquotted and stored in 60 mL jars in a blast freezer at –40 °C until used (usually within four weeks). The hydrolysate was mixed with 3,5-dinitrosalicylic acid (DNSA, Sigma, St. Louis, MO) and the reaction product of maltose-DNSA was measured using a spectrophotometer at 540 nm [9].

The maltose concentration was determined using a standard curve of maltose content vs. absorbance. The RDS, SDS, RS, and percent decrease in digestibility were calculated as follows:

$$\%RDS = \frac{D-E}{F} \times 100$$

D = mg maltose produced on digestion of cooked debranched modified starch at 1 h time interval

E = mg maltose produced on digestion of cooked debranched modified starch at 0 h time interval

F = mg of total starch

$$\%SDS = \frac{G-H}{I} \times 100$$

G = maximum mg maltose produced on digestion of cooked debranched modified starch at a certain time interval after which no further increases are noticed

H = mg maltose produced on digestion of cooked debranched modified starch at 1 h time interval

I = mg of total starch

$$\%RS = \frac{J-K}{J} \times 100$$

J = mg of total starch

K = maximum mg maltose produced on digestion of cooked debranched modified starch at a certain time interval after which no further increases are noticed

$$\%\text{Decrease in digestibility} = \frac{A-B}{A} \times 100$$

A = mg maltose of cooked non-debranched starch control at a particular time interval during incubation with amylase

B = mg maltose of cooked debranched modified starch at that time interval

3.5 Particle size analysis

A model LS 230 particle size analyzer (Coulter Corporation, Miami, FL) with a small volume module was used to determine the particle size. Water was used as dispersion medium in the analyzer. Starch sample (1 g) was mixed with 2 mL of water and vortexed for 5 min. Water was pumped into the small volume module and drained, this was repeated three times. A small volume of modified starch sample was introduced into the instrument until 45% obscuration or 8–12% PIDS (Polarization Intensity Differential Scattering) had been reached. A spectrum of particle sizes present was measured and analyzed.

4 Results and Discussion

4.1 Degree of Debranching

Various factors affect the processes for making reduced digestibility starches, including degree of debranching and temperature of cooling [6]. The change in the concentration of debranched starch was followed by gel permeation chromatography (GPC) and iodine affinity. Fig. 1 shows a typical gel permeation chromatogram of the non-debranched starch, and debranched starch after 24 h of debranching using 10% pullulanase. The appearance of two lower molecular weight peaks (peak 2 and 3) in the chromatogram after 24 h of debranching indicated that the chains were debranched. After debranching, hydrolysates of the original peak (peak 1) of the non-debranched starch elute at higher volume (peak 2 and 3).

Peak 2 probably represents amylopectin which has been partially debranched and linear long chain amylose molecules. Peak 3 represents short chain amylose. Peak 3 was used as an index of debranching [8]. Fig. 2 shows the
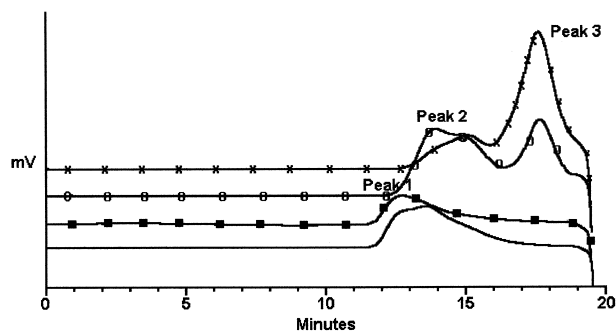
Fig. 1. A typical chromatogram of waxy and non-waxy starch before debranching and after debranching with 10% pullulanase after 24 h. Non-debranched non-waxy (–), non-debranched waxy (■), 24 h debranched non-waxy (**0**), and 24 h debranched waxy (**x**).

increase in the third peak during debranching. A rapid increase in debranching during the first 4 h occurred, followed by a slow increase up to 24 h. Similar results were obtained in a previous study [6].

Similar results were obtained using the iodine affinity method to determine debranched fragments in peak 3 (Fig. 3). Linear regression analysis indicated a positive correlation between iodine affinity and the peak 3 in the chromatogram of waxy and non waxy starches during debranching, with r^2 of 0.98 and 0.99, respectively.

Therefore, iodine affinity can also be used for determining the production of branch chain fragments during debranching. All the branches released in the first eight hours of debranching apparently were long enough for forming starch-iodine complexes.
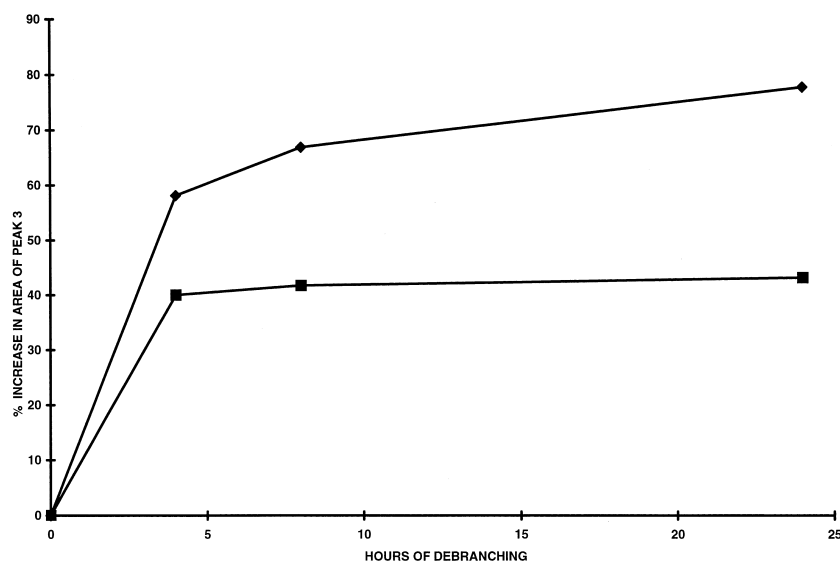

Fig. 2. % increase in the area of Peak 3 of gel permeation chromatogram (Fig. 1) of debranched waxy (♦) and non-waxy (■) starch at various time intervals during debranching with pullulanase (Pooled standard error 0.42).
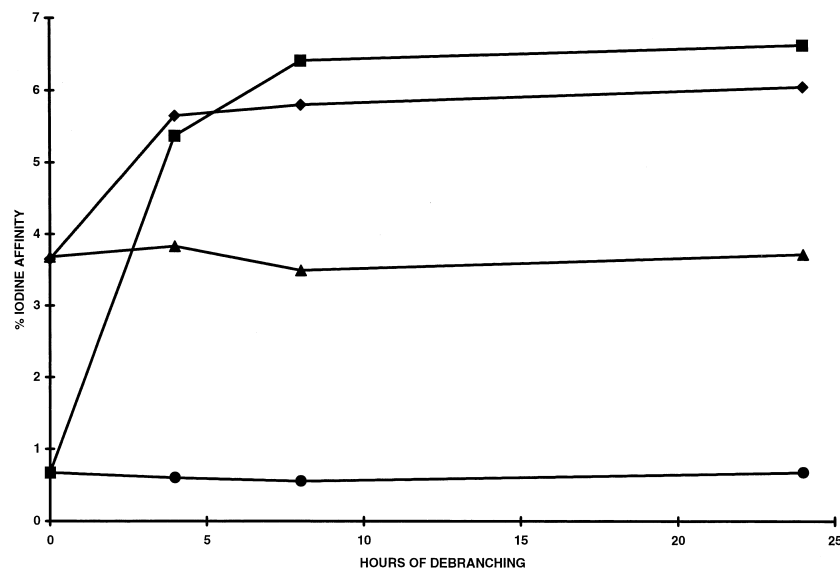

Fig. 3. % iodine affinity of waxy (■) and non-waxy (♦) starch during various time intervals of debranching with pullulanase. Legend: non-debranched non-waxy (▲), and non-debranched waxy (●) starch (Pooled standard error 0.10).

After 8 h no significant increases in iodine affinity are observed. Based on iodine affinity and GPC, a high level of debranching had occurred during first few hours of debranching.

4.2 Effect of cooling debranched starch on physical properties of resulting material

4.2.1 Gel formation (water holding capacity and texture)

All debranched starch solutions were heated in an autoclave at 121 °C for 30 min to stop any further enzymatic activity and to unwind any double helices, melt the crystallites, and/or unretrograde starch which might have formed during the initial heating and incubation at 57.5 °C for debranching. Visible precipitates formed immediately after removing the debranched solution from the autoclave. The precipitates dissolved on mixing while the solution was hot. As the cooling progressed, increases in turbidity and precipitate formation were noticed. *Miles* et al. [10] and *Doublier* and *Choplin* [11] have shown that amylose solutions of low concentration (1.6–2.4%) become turbid during the early stage of amylose gelation. The increase in turbidity was attributed to a phase separation into polymer-rich and polymer-deficient regions. This may be followed by network formation. The rate and extent of network formation increases with increasing molecular sizes [12]. *Gidley* and *Bulpin* [12] described these processes in a phase diagram showing the effect of cooling aqueous synthetic amylose solutions. Short chain amyloses (DP < 110) were found to precipitate at all concentrations up to 5%. Amylose of DP 40–65 gave fine, dense precipitates while precipitates of DP 90 and 110 are less dense. For chains of intermediate length (250 < DP < 660), both gelation and precipitation are observed while long chain amylose (DP > 1100) are found to form

gel on cooling. *Gidley* and *Bulpin* [12] formulated a reasonable explanation for the observed effects based on the relative importance of chain alignment and cross-linking in cooled aqueous solutions. Thus, for long chains, extensive cross-linking occurs which results in the formation of a macromolecular network eventually resulting in gelation. If the chain length over which these interactions occur is substantially smaller than the total chain length, then more than two regions within a single chain could be involved in separate interactions, thereby leading to a cross-linked network structure. Conversely, if the total chain length is not substantially longer than the interacting chain length, then extensive cross-linking will not occur and chain alignment will predominate, a process which, if followed by lateral aggregation, would eventually lead to precipitation (following extensive aggregation). Lateral aggregations of shorter chains are due to formation of double-helices. These helices further aggregate leading to formation of ordered crystalline arrays which precipitate out of solution. In general, precipitation is favored by shorter chain lengths, lower concentration and slower cooling rates and gelation is favored by longer chain lengths, higher concentrations and faster cooling rates.

Gel formation is an important parameter to consider in developing a product suitable for use in food. Formation of firm gel might make the starch product suitable for use in textured foods while a poorly gelled product or one which does not form a gel might make it suitable for use in drinks. Gels were only formed in samples that were not agitated during cooling. We observed poor gel formation in case of waxy starch (Figs. 4 and 5). The water holding capacity measures the % weight of the total starch suspension as a gel which holds the water on centrifugation excluding supernatant (Fig. 4). The water holding capacity increased from 8% at 0 h to 35% at 4 h cooling for waxy
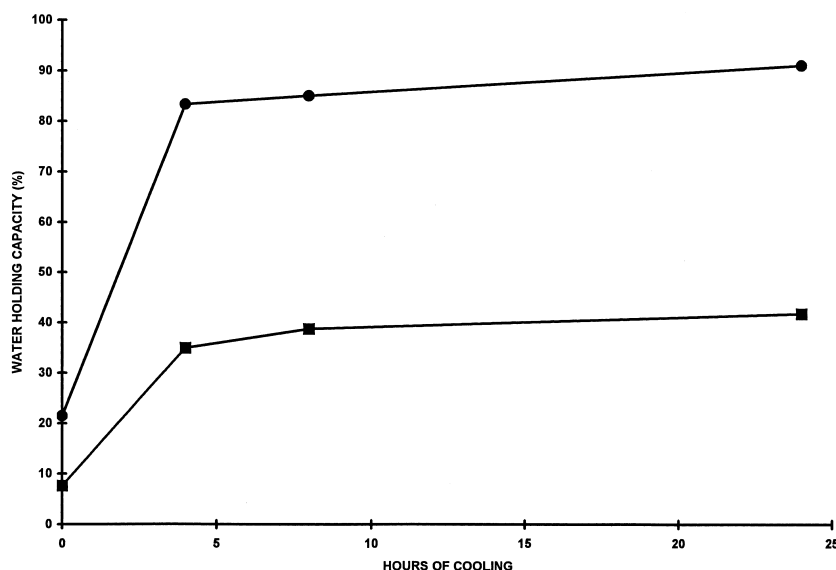

Fig. 4. Effect of cooling (1 °C) of debranched waxy (■), and non-waxy (●) on starch water holding capacity (Pooled standard error 0.71).
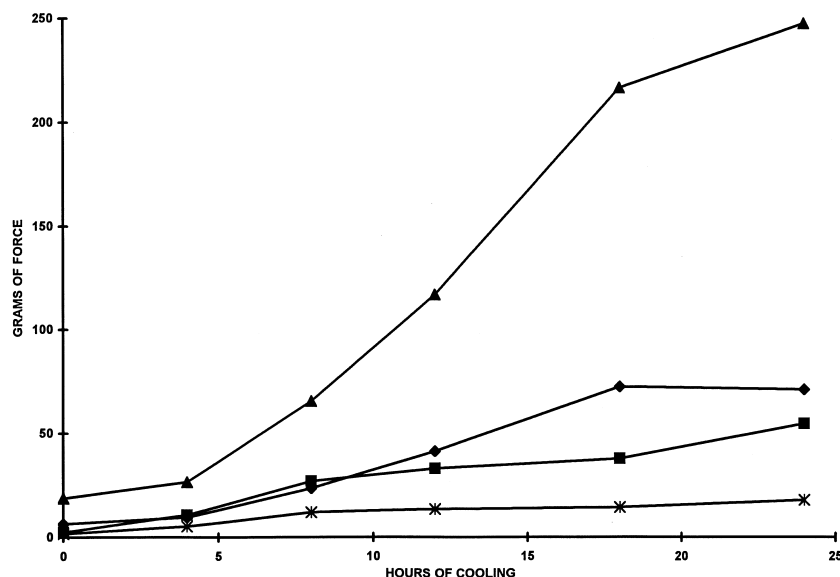
Fig. 5. Effect of cooling (1 °C) debranched waxy and non-waxy starch on hardness (■ waxy and ▲ non-waxy) and adhesiveness (✳ waxy and ◆ non-waxy) of the gel (Pooled standard error 2.26).

cooled debranched starch while that of non-waxy starch increased from 22% at 0 h to 84% at 4 h and did not change after that (Fig. 4). Non-waxy starch held more water than waxy starch. No increases in hardness or adhesiveness took place in the first 4 h of cooling non-waxy debranched starch (Fig. 5). The hardness of the debranched waxy starch increased from 10 g to 45 g of force but adhesiveness did not increase from 0–24 h of cooling (Fig. 5). The non-waxy starch continued to increase in hardness and adhesiveness up to 245 and 70 g of force beyond 24 h, respectively (Fig. 5). This high textural strength and water holding capacity is probably due to longer chains in non-waxy starch. Amylose molecules with a high degree of polymerization can be involved in more than one double helix formation; more than one cross-link (a junction zone) gives a network structure or gel [13]. This cross-linking usually occurs in the more than 100 glucose residue regions in the amylose chain, not in amylopectin [12, 14]. In the case of waxy starch, due to absence of longer chain amylose and greater abundance of smaller chains (< DP 90) [15, 16] aggregation resulting in precipitation and formation of denser but smaller precipitates is favored over formation of junction zones. This could lead to formation of weak gel or precipitates as observed for debranched waxy rice. The initial rise in gel strength follows probably establishment of a three-dimensional network and involves increasing amounts of chains in sol fraction into the gel. The subsequent slower rise would then probably be caused by further rearrangement of cross-links and lateral chain aggregation resulting in net decrease in the length of elastically active chains. This phenomenon of continued increase in hardness and adhesiveness values (Fig. 5) is called gel aging or annealing, similar to that observed for long chain (DP 2500 and 2800) synthetic amylose [14]. Continued increase in

crystallinity was also observed beyond 1500 min of cooling amylose solution at various concentration by quenching at 32 °C [17]. Crystallinity and gel strength is also strongly related to concentration [11, 17] which should be carefully studied when developing a product.

4.2.2 Particle size

The particle size of the debranched starch changed during cooling (Fig. 6). The non-waxy debranched starch had a particle size of 28 μm at 0 h, which increased to about 45 μm after 4 h of cooling. In the case of debranched waxy starch, particle size decreased from 70 μm to about 4 μm after 4 h of cooling. *Gidley* and *Bulpin* [12] also observed smaller size precipitates for shorter amylose chains DP (<110) due to formation of aggregates, as described earlier, while longer chains formed gels and bigger precipitates. Non-debranched starch did not change in particle size during cooling. The particle size remained around 15 μm for non-waxy starch and 11 μm for waxy starch.

4.2.3 Effect of time of cooling and agitation on digestibility of debranched starch during cooling

Debranched non-waxy starch that was cooled for 12 h without agitation decreased in digestibility by 59%; longer cooling periods did not change the degree of digestibility. In contrast, with agitation digestibility of the debranched non-waxy starch decreased by 42% after 24 h (Fig. 7). Debranched waxy starch made without agitation decreased in digestibility by 58% after 8 h of cooling, but with agitation digestibility decreased by 35%. These results suggest that stirring prevented or slowed the rate of formation of crystalline structures or the double helices from aligning with each other. If a reduced digestibility
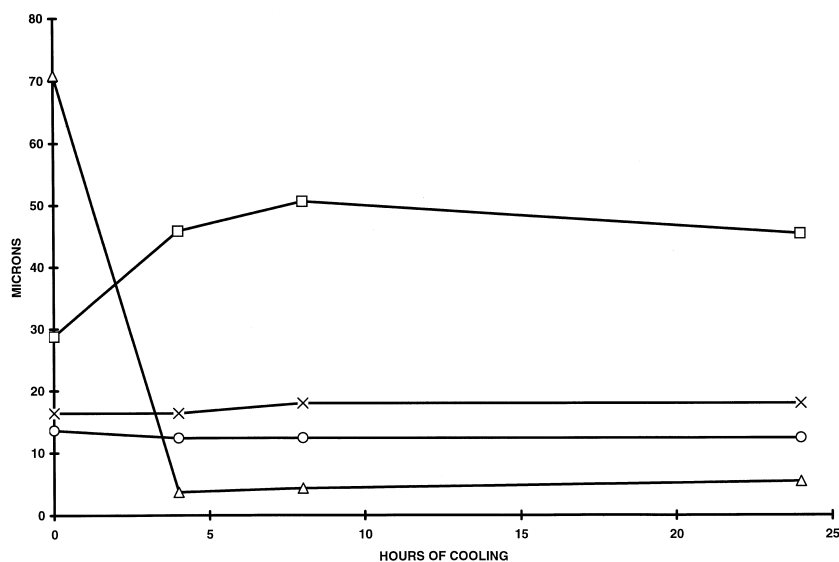
Fig. 6. Effect of cooling (1 °C) debranched waxy and non-waxy starch on particle size. Legend: waxy non-debranched (○), non-waxy non-debranched (×), waxy debranched (△), and non-waxy debranched (□) starch (Pooled standard error 2.08).
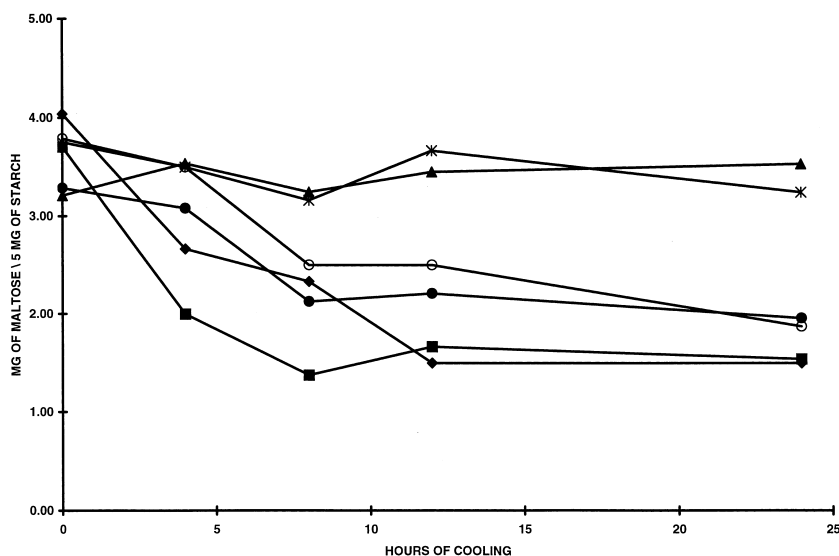

Fig. 7. α-Amylase hydrolysis of stirred and non-stirred waxy and non-waxy starch during cooling. Waxy non-debranched (✻), non-waxy non-debranched (▲), waxy debranched with stirring (●), non-waxy debranched with stirring (○), waxy debranched without stirring (■), and non-waxy debranched without stirring (◆) (Pooled standard error 0.06).

product was desired, then the debranched starch should be held for 12–24 h in storage without agitation at 1 °C. Beyond that there are no further decreases in digestibility. In contrast, the gel hardness of non-waxy starch continued to increase beyond 24 h which suggests that the texture of the cooled debranched starch does not effect digestibility. The digestibility of non-waxy and waxy starch that was not debranched did not decrease during 24 h of cooling, suggesting extensive branching that prevented the starch chains from coming together and forming crystalline structures.

4.2.4 Effect of cooling debranched non-waxy and waxy starch from 0 to 24 h time interval followed by freezing on digestibility

Figs. 8 (non-waxy) and 9 (waxy) show the effect of the time interval of cooling at 1 °C followed by freezing at

−40 °C. Cooling prior to freezing had no effect on digestibility. We observed in the previous experiment (Fig. 7) that if only cooling is applied, it takes about 12 h before no further changes in digestibility are observed. Cooling for various time interval from 0–24 h followed by freezing did not exhibit large differences in digestibility for both debranched non-waxy and waxy starch (Figs. 8 and 9). There was no statistical difference (Fishers LSD test α = 0.05) between debranched non-waxy samples stored at various cooling time intervals before freezing at −40 °C over all the digestibility intervals except the 8 h digestibility measurement. Only the debranched non-waxy samples stored at 0 h and 12 h of cooling were significantly different (Fishers LSD test α = 0.05). The average RDS, SDS, and RS was 33.7%, 53.2%, and 13.1% for debranched non-waxy starch (Fig. 8) over all time intervals. In a previous study [6], RDS, SDS, and RS was 26%, 30% and 44% for 10% non-waxy starch debranched for

24 h using 10% pullulanase as used for this study followed by cooling and measurement of digestibility. The difference was that samples prepared in this study were frozen, precipitates were washed, dried, and milled to 80 mesh before measurement of digestibility. The differences are probably due to differences in processing methods.

There was no statistical difference (Fishers LSD test $\alpha = 0.05$) between waxy debranched samples stored at various cooling time intervals before freezing at –40 °C over all the digestibility intervals except the 1, 2, and 24 h digestibility measurement. These differences did not follow any trend. The average RDS, SDS, and RS was 51.7%, 18%, and 30.4% for debranched waxy starch (Fig. 9) over all time intervals. In a previous study [6],

RDS, SDS, and RS was 16%, 34% and 50% for 10% waxy starch debranched for 24 h using 10% pullulanase as used for this study followed by cooling and measurement of digestibility. There is a 69% increase in RDS and both the SDS and RS decreased by 47% and 32%. It seems that freezing, washing of precipitates, drying, and milling to 80 mesh before measurement of digestibility reduced or broke down the crystalline structure formed during cooling.

If it is desired to make a starch with high amounts of SDS using debranching followed by freezing and drying, then non-waxy starch would have to be used. It results in the highest amounts of SDS including the results from previous study [6] for both waxy and non-waxy starch. The highest amounts of SDS was 44% using waxy starch de-
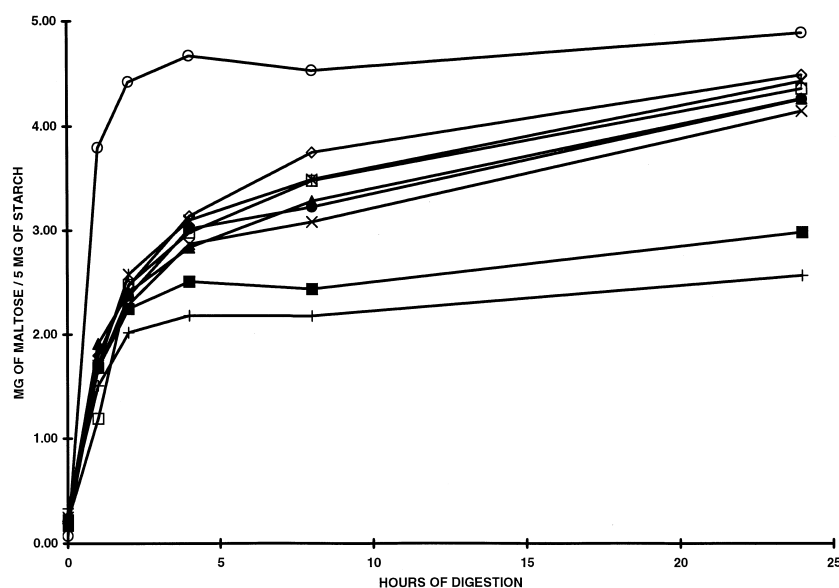

Fig. 8. Effect of cooling non-waxy debranched starch at 1 °C for 0, 4, 8, 12, 18 and 24 h followed by freezing at –40 °C, thawing, drying and milling on the rate of digestibility. Cooling time intervals, 0 h (◇), 4 h (□), 8 h (▲), 12 h (×), 18 h (✳), 24 h (●), opta® resistant starch (■), novelose® resistant starch (+), and freshly cooked non-waxy non-debranched starch (O) (Pooled standard error 0.12).
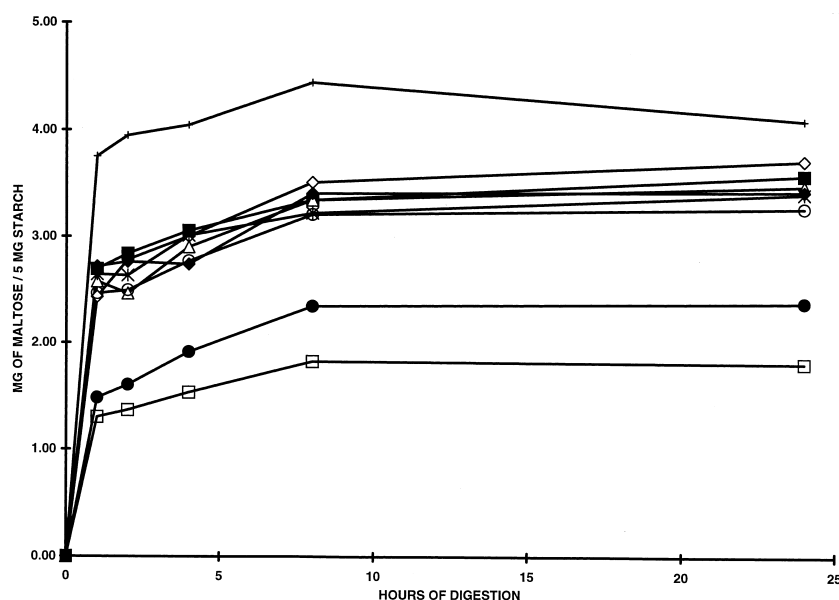

Fig. 9. Effect of cooling waxy debranched starch at 1 °C for 0, 4, 8, 12, 18 and 24 h followed by freezing at –40 °C, thawing, drying and milling on the rate of digestibility. Cooling time intervals, 0 h (◇), 4 h (■), 8 h (△), 12 h (○), 18 h (✳), 24 h (◆), opta® resistant starch (□), novelose® resistant starch (●), and freshly cooked waxy non-debranched starch (+) (Pooled standard error 0.11).

branched for 4 h and cooled for 24 h and maximum RS (50%) was for waxy starch debranched for 24 h [6]. We had expected that freezing the debranched starch solution at −40 °C without cooling should have inhibited the starch chains from coming together and forming semi-crystalline structures and very little decreases in digestibility would take place, at the very least there would be increased formation of SDS. As the cooling time increased followed by freezing, we thought that there would be a decrease in digestibility. We had hypothesized that the viscosity would increase slowly as cooling time at 1 °C increased as compared to freezing, therefore allowing starch chains to come together, resulting in higher decreases in digestibility during cooling. These differences were not noticed which could be due to differences in processing methods. We also studied the effect of freezing debranched starch solution at various temperatures (−2, −10, and −40 °C) immediately after debranching and autoclaving on the decrease in digestibility (data not shown). We found no statistical difference in digestibility between samples stored at various freezing temperatures (Fishers LSD test $\alpha = 0.05$). Storing the debranched starch at various freezing temperatures would cool the debranched starch at different cooling rates but this did not effect the digestibility. The debranched starch made for the present study was dried and milled before testing for digestibility. The milling could significantly effect the digestibility.

4.2.5 Effect of particle size on digestibility

Non-waxy debranched starch was cooled for 24 h, thawed, washed and dried. The particle size measured using the Coulter particle size analyzer was 412, 300, 143, and 118 µm for the dried starch milled using 20, 30,

60 and 80 mesh screens in a Wiley mill. On regression between percent maltose produced after 24 h of digestion (Fig. 10) and the particle size determined with the Coulter particle size analyzer, the r^2 (coefficient of correlation) was 0.98, which suggests a high degree of correlation. The digestibility is directly related to the particle size, the larger the particle the lower the digestibility. When developing slowly digestible or resistant starches for human nutrition, the particle size of the product has to be evaluated.

4.2.6 Comparison of decreased digestibility starches described here with commercial resistant starches

The resistant starches made by *Iyengar* et al. [18] from Opta Food Ingredients, and *Chiu* et al. [8], and *Henley* and *Chiu* [19] from National Starch and Chemical Corporation differ from our product. Their products are more resistant to digestion (Figs. 8 and 9). Crystalean®, the product made by Opta Food Ingredients is starch dispersed in an aqueous medium and then subjected to extended incubation at elevated temperature (60–120 °C for a period ranging from 5–10 h for the retrogradation to occur) followed by incubation at lower temperatures (about 4–20 °C) for 0.5 to 4 days. The retrogradation of amylose is retarded if amylopectin is present in the starch. The process can be accelerated by enzymatic conversion of amylopectin to amylose before retrogradation, by use of debranching enzymes; such as pullulanase or isoamylase; or by partial hydrolysis by α-amylase. This retrograded polysaccharide is then subjected to the hydrolysis by enzymes or acid to reduce amorphous regions and to produce a starch that is essentially free of amorphous regions and 90% crystalline. The resulting residue is washed and dried to make enzyme modified retrograded
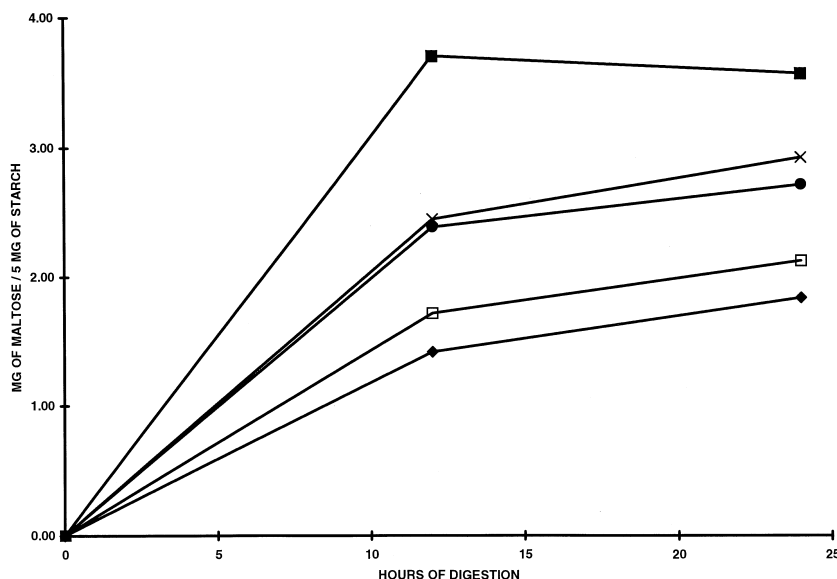

Fig. 10. Effect of particle size of debranched freeze/thaw dried milled starch on rate of digestibility of non waxy rice starch. 20 mesh (◆), 30 mesh (□), 60 mesh (●), 80 mesh (×), and freshly cooked (■) non-debranched freeze/thaw dried starch (Pooled standard error 0.12).

amylose that is an insoluble low calorie starch product. The patents issued to *Chiu* [8], and *Henley* and *Chiu* [19] describe a process for making amylase resistant starch from high amylose starch. The method consists essentially of gelatinizing a slurry of starch that contains amylose in an amount greater than 40%, treating the gelatinized starch with a debranching enzyme for sufficient time to effect essentially complete debranching, deactivating the enzyme, and isolating the starch product by hot air drying, extrusion or crystallization by addition of salt. In comparison, our starting material, rice starch, has a lower amylose content than the corn starch used in these commercial products. We found that incomplete debranching and cooling at 1 °C was required to form slowly digestible starches. Freezing non-waxy debranched starch followed by isolation, drying and milling of starch makes a product high in SDS. Both processes [8, 18, 19] used to make resistant starches manufactured commercially require complete debranching to make resistant starch.

5 Conclusion

The development of SDS products from debranched starch is a challenge. The previous study [6] outlined the effect of concentration of pullulanase enzyme and temperature of cooling on formation of SDS. Results indicated that a product containing 50% SDS could be developed by controlling the debranching process and the temperature of cooling. This study indicates that the debranched non-waxy starch forms a high strength gel while waxy starch stays liquid. It seems that freezing, washing of precipitates, drying, and milling to 80 mesh before measurement of digestibility reduced the crystalline structure formed during cooling, therefore drying does not maintain the same digestibility as obtained before freezing followed by drying. The functional properties (texture and water holding capacity) are also all lost during the drying process, they are reversed after autoclaving at 121 °C followed by cooking. Due to reversible nature of these properties at autoclaving temperatures, cooling will have to be done after processing to restore the functional properties and also reduced digestibility. For distribution of this material to the end user, starch could be debranched optimally, followed by drying. On receipt of the dry product by the end user it could then be hydrated, heated, and cooled to cause retrogradation at optimum conditions described in this and the previous study [6] before use. We found that non-waxy starch debranched for 24 h followed by cooling, freezing and drying resulted in a product with 50% SDS but it does not have any functional properties. This product could be used in a suspension or into dried product to provide SDS. The effect of interaction with protein and fats will have to be studied in a drink formulation where we will be heating the de-

branched starch product. Work is currently underway to develop SDS which is heat stable.

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